THE BACTERIAL DISEASES OF PLANTS:

A CRITICAL REVIEW OF THE PRESENT STATE OF OUR KNOWLEDGE.

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I.

It is scarcely fourteen years since Dr. Robert Hartig declared that there were no diseases of plants due to bacteria.[1] Two years later Dr. Anton de Bary, unquestionably one of the most learned and critical botanists the world has ever known and the foremost student of cryptogamic plants, expressed the belief that bacterial diseases of plants were of rare occurrence, and suggested as a partial explanation the fact that the tissues of plants generally have an acid reaction.[2] In his *Vorlesungen über Bacterien*, published in 1885, he expresses much the same opinion,[3] and cites only four diseases, viz., Wakker's hyacinth disease, Burrill's pear blight, Prillieux's rose red disease of wheat grains, and the wet rot of potatoes, described by Reinke and Berthold. Concerning the first of these four diseases he says: "Successful infection experiments and exact study of the life history of the bacterium are still wanting." Respecting the second he contents himself with briefly summarizing the statements made by Prof. Burrill. Of Prillieux's micrococcus he says: "Its importance as a cause of disease cannot be determined with any certainty from the brief account. It

[1] "Für die Krankheitsprocesse der Pflanzen kommen sie durchaus nicht in Frage, etc." Hartig: (*1*) *Lehrbuch der Baumkrankheiten*, 1882, p. 27.

[2] "Bacteria parasitic on plants have scarcely ever been observed, a fact to which R. Hartig has already drawn attention. One reason for this may be that the parts of plants have usually an acid reaction." De Bary: (*2*) *Vergleichende Morphologie und Biologie der Pilze Mycetozœn und Bacterien*, 1884, p. 520; English ed., p. 481.

[3] "According to the present state of our knowledge parasitic bacteria are of but little importance as the contagia of plant diseases. Most of the contagia of the numerous infectious diseases of plants belong to other animal and plant groups, principally, as already noted, to the true fungi." De Bary: (*3*) *Vorlesungen ueber Bacterien*, 1885, p. 136.

may turn out to be only secondary, appearing as a saprophyte in consequence of injuries previously received." Concerning the wet rot of potatoes he states that ordinarily it is a secondary phenomenon following the attacks of the parasitic fungus *Phytophthora infestans*, but admits that exceptionally potato tubers may become wet rotten without the presence of *Phytophthora*, and that "the above named observers succeeded in producing the appearance of wet rot in sound potato tubers by inoculations with their bacteria; in agreement with which stands a recent experiment of van Tieghem, who succeeded in totally destroying living potato tubers by means of *Bacillus amylobacter* when he introduced this into the interior of the tuber and maintained the same at a high temperature (35°)."

In the second edition of his *Lehrbuch*, published in 1889, Dr. Hartig modified his statements somewhat, expressing essentially the same opinions as de Bary. The yellow rot of hyacinths is recognized as a bacterial disease, although rather doubtfully in as much as it is said not to attack sound, well-ripened bulbs, under normal conditions, but only when they have received wounds or been attacked by fungi, especially by a hyphomycete which is said to be an almost constant accompaniment of the rot. The wet rot of potato tubers is admitted to the list, but with the statement that it is mostly a secondary matter, following the rot of stem and cells due to *Phytophthora infestans*. One other bacterial disease is mentioned, viz., pear and apple blight, with the suggestion, however, that it may have been erroneously attributed to bacteria, since the fungus *Nectria ditissima* produces in the bark numerous little bacteria-like gonidia.

Such was the general opinion on this subject down to within less than a decade. Even to-day the majority of well educated botanists would find nothing to contradict in the statement that there are very few diseases of plants distinctly attributable to bacteria. As a matter of fact, however, there are in all probability as many bacterial diseases of plants as of animals.

Various explanations have been advanced to account for this freedom or supposed freedom of plants from bacterial parasitism. As we have already seen, de Bary was inclined to ascribe

it in good part to the acid reaction of vegetable tissue. Dr. Hartig's view is best expressed in his own words:[4] "Whereas the processes of decay, and most of the infectious diseases of man and animals, may be traced to bacteria, the plant organism is protected against them by the peculiarity of its structure, and especially by the absence of circulatory channels for conducting the nutrient fluids which could serve to distribute any lowly organisms which might happen to be present in the food. It is only by means of the vessels and intercellular spaces that they can distribute themselves in any great numbers in the body of the plant, for in other cases they have to pass through the cellulose or woody cell walls, which offer great resistance to their attack. In addition to this, the vegetable juices, most of which show an acid reaction, are unfavorable to their growth. As a matter of fact, bacteria have hitherto been found only in the tissues of plants whose cells are parenchymatous in character and possessed of very delicate walls, as for instance, bulbs and tubers."

For several years Ph. van Tieghem experimented with one or more, probably several, bacteria, called by him *Bacillus Amylobacter* and believed to be the specific agent in the decomposition of cellulose. In 1879,[5] he stated that all the cells of all plants are equally dissolved by it in the meristematic stage but that as soon as the tissues have become differentiated profound differences are noticeable. The cellulose of many plants is dissolved by it but that of mosses, sphagnums, hepatics, lycopods, fern leaves, and stems and leaves of phanerogamous aquatics proved resistant. This behaviour of water plants is "une nécessité d'existence." In 1884,[6] he made a number of additional similar statements. The tubers of the potato, the seeds of beans (first swelled in water and then inoculated directly into the substance of the cotyledons), and the fruits of cucumbers and melons rotted quickly when infected with this organism. Inoculated leaves of Crassulaceæ and stems of Cac-

[4] Hartig: *Lehrbuch.* 2nd. Edition. English translation, p. 37.

[5] Van Tieghem: (*4*) Sur la Fermentation de la Cellulose. *Bull. de la Soc. Bot. de France*, 1879, pp. 25 to 30.

[6] Van Tieghem: (*5*) Développement de l'Amylobacter dans les plantes à l'état de vie normale. *Ibid.*, 1884, pp. 283–287.

taceæ resisted until plunged under oil when they decayed quickly. Aquatics resisted: "By means of a Pravaz syringe I have injected a drop full of the spores of Amylobacter into the lacunary system of several submerged aquatics (Vallisneria, Helodea, Ceratophyllum) but always without result. The plant remained healthy in all its parts."

These papers of van Tieghem are often cited, but they have little substantial value. Undoubtedly he believed that he was experimenting with pure cultures, or, at least, that the results obtained were due to *Bacillus amylobacter*, but such is, to say the least, very improbable. *B. amylobacter* is now believed to be strictly anaerobic, and incapable of any action on cellulose.[7]

More recently Julius Wiesner has divided all plants into two classes, ombrophobic and ombrophylic plants, according as they are or are not readily injured by prolonged rains or exposure to stagnant fluids.[8] His experiments show that the aerial parts of some plants rotted very quickly when exposed to continuous artificial spray while similar parts of other plants proved very resistant, remaining sound for weeks (62 days in case of *Tradescantia guianensis*). The same contrast was observed when leaves of the two sorts of plants were placed in stagnant water, the former lost their turgor and rotted in a few days, the latter proved much more resistant. Many land plants have this power of resistance and all water plants, also all underground parts, even the roots of plants having very susceptible foliage. As additional confirmation Wiesner states that when meat infusions are left to themselves they always decay much sooner than when fragments of ombrophylic plants are placed therein. Ombrophobic plants in water or meat infusion also decay less rapidly when mixed with fragments of ombrophylic plants than when left to themselves. This decay is more rapid in the dark than in light, especially

[7] Prazmowski: (*6*) *Untersuchungen ueber die Entwickelungsgeschichte und Fermentwirkung einiger Bacterien-Arten.* Leipzig, 1880, pp. 23–37.

[8] Wiesner: (*7*) Ueber ombrophile und ombrophobe Pflanzenorgane, *Sitzungsb. K. Ak. d. Wissenschaften*, Math.—Naturw. Classe. Wien., 1893, Bd. 102. Abt. I, pp. 503–521. See also Wiesner: (*8*) Pflanzenphysiologische Mittheilung aus Buitenzorg (III). Ueber den vorherrschend ombrophilen charakter des Laubes der Tropengewächse. *Ibid.*, 1894, Bd., 103, pp. 169–191.

bright light. The foliage of ombrophylic plants is easily wetted; that of ombrophobic plants is as a rule not readily wetted, being usually protected by bloom or some other device for warding off water. When ombrophobic plants are not protected in some such manner, decay is remarkably rapid. In general if the leaves of a plant are readily wetted, it may be assumed that they are ombrophylic, but there are exceptions, e. g. the potato and tomato. Roots of all plants are extraordinarily resistant. In most plants middle aged leaves are least susceptible to decay but in the potato the youngest leaves resist best. Old leaves lose this power of resistance. Sometimes this resisting power is variable in different individuals of the same species, depending on the conditions under which they have been grown. Curiously, all plants of shady, damp places are ombrophobic, if they possess leaves which are not readily wetted, e. g. Impatiens. The more the parts of a leaf are divided the quicker the decay. The green parts of the following plants are mentioned as particularly susceptible to bacterial decay: *Solanum tuberosum*, *Lycopersicum esculentum*, *Xeranthemum annuum*, *Impatiens nolitangere*, *Chenopodium album*, *Veronica buxbaumii*, *Viola arvensis*, and *Taraxacum officinale* (from sunny, dryplaces) *Mimosa pudica*, *Pisonia alba*. The following plants were found to be very resistant: *Ranunculus aquatilis*, *Lemna minor*, *Lysimachia nummularia*, *Begonia magnifica*, *Tradescantia zebrina*, *T. guianensis*, *Selaginella sp.* (from green house), and *Scolopendrium officinarum*. Among underground organs the roots of the yellow beet proved most resistant. The author's general conclusion from these experiments is best expressed in his own words: "It can now be stated as highly probable that the power of ombrophilous organs to resist rain for months is referable chiefly to the fact that antiseptic substances are produced in the tissues of the organs." These experiments are interesting but seem to have been performed in a rather crude way. The relative rapidity of decay was determined by appearance and the sense of smell and the organisms inducing this decay were undetermined. These experiments should be repeated and extended by Dr. Wiesner

or by some bacteriologist, using pure cultures and plant juices which have been sterilized by filtration.

Dr. Russell's experiments[9] were made a year earlier than than those of Wiesner and have the merit of being properly performed, i. e. with sterile juices and pure cultures so that the conditions under which the experiments were made can be reproduced by other investigators. They are, however, too limited in number to afford any basis for a general conclusion. He found that Canna juice, sterilized by filtration, exerted no appreciable germicidal effect on any of the following species: Kiel-water bacillus, *B. lactis-ærogenes*, *B. coli-communis*, *B. megaterium*, *B. prodigiosus*. Experiments with *B. megaterium*, *B. butyricus*, *B. coli-communis*, *B. pyocyaneus*, and *Streptococcus pyogenes*, using as a culture medium root-pressure juice obtained under sterile conditions from the severed stem of lima beans and Pelargoniums led to a similar conclusion and to the enunciation of the following general statement: "vegetable cell juices, aside from their acid reaction, are entirely powerless against bacteria, and do not possess any germicidal properties like the blood serum of animals."

The old view that plants are not subject to the attacks of bacteria simply because their tissues are acid was shaken by the discovery that some bacteria grow very well in acid media, and was thoroughly upset by the discovery that the juices of some parts of many plants are alkaline. In all probability plants like animals require a delicate balance between acid and alkaline and a continual change from one side to the other for the carrying on of the life processes. Three things at least are certain (1) It will not do to assume that all parts of a plant are acid because some part of the parenchyma shows a strongly acid reaction; (2) It cannot be stated that any given microorganism will thrive only in alkaline media until this fact has been determined by direct experiment; and (3) Many bacteria, perhaps all, are alkali producers and capable, if they can gain any foothold whatever, of slowly changing an unsuitable acid medium into one more alkaline and better adapted to their use.

[9] Russell: (*9*) *Bacteria in their Relation to Vegetable Tissue.* Thesis. Johns Hopkins University. 1892, 8vo. p. 41.

Wiesner's hypothesis is somewhat different. It has been known for some time that various essential oils and other vegetable products, e. g. thymol, salicylic acid, benzoic acid, tannin, quinine, oil of cinnamon, oil of peppermint, etc., exert a powerful restraining influence on the growth of many bacteria, and it is not improbable that a great variety of bactericidal and protective substances occur in plants. On the other hand there may be and probably are bacterial parasites capable of thriving in the very plants which Wiesner found most resistent to continuous spray, to the saprophytic bacteria of stagnant water, and to those of decaying meat infusions, the exact conditions under which any given microorganism will thrive being determinable only by experiment. It must also be remembered that the physiological requirements of bacteria often become profoundly modified to suit changed environments, and that all parasites have undoubtedly descended from saprophytic forms. Prof. Wiesner has, however, opened up a very inviting field and its further investigation by some careful experimenter, trained in bacteriological methods, might lead to very interesting discoveries.

Most of the recent books on vegetable pathology devote a chapter to the bacterial diseases of plants, but these books have not been written by bacteriologists and consequently the statements given are usually very meager and unsatisfactory, and forcibly illustrate the fact that no one can write acceptably on a subject with which he is not familiar, not even if he possesses a logical mind and has read all the "authorities." Excepting Prof. W. Migula, who reviewed the subject briefly but somewhat carefully in 1892,[10] and Dr. H. L. Russell, who gave a brief summary in tabular form the same year at the end of his Thesis,[11] no one seems to have gone over the field critically since de Bary's time, although there is now a considerable body of literature. It is proposed, therefore, in the following pages to examine the literature of this subject from the standpoint of the

[10] Migula: (***10***) *Kritische Uebersicht derjenigen Planzenkrankheiten, welche angeblich durch Baktrien verursacht werden.* Semarang. Midden-Java. 1892. Exp. Sta.

[11] Russel: l. c., pp. 36–41.

modern bacteriologist, sifting as far as possible the wheat from the chaff, and arranging all in an orderly way for convenient reference. The utility of such a piece of work, if well done, can scarcely be questioned, since it must set into sharp relief the gaps in our knowledge and tend to stimulate further research.

The work of the early investigators already mentioned was done before the perfection of modern methods of bacteriological research, and in a time of general scepticism which some of us well remember. It is therefore in no way discreditable that many of their conclusions should be found untenable when tested by the more rigid requirements of the science of to-day. They worked under great difficulties and did as well as could be expected even of men of genius, better, indeed, than many of us would have done. Certainly, as pioneers in a difficult field they deserve great credit.

As much cannot be said for some of the more recent workers who with every opportunity in the way of literature, including numerous manuals of bacteriology, and with laboratory facilities for learning the fundamentals of bacteriological research on every hand in every land, have been content to publish second and third class work, exactly like that preceeding the discoveries of Pasteur and Koch and the development of modern methods. One might suppose these people to have been in a deep sleep for the last twenty years, they take so little note of what has been going on. I shall have frequent occasion to consider papers of this class in the course of these pages and shall not fail to point out their worthlessness, to discourage imitators, if for no other reason. It goes without saying that such publications do not advance science, nor in the end in any way contribute to the reputation of the individual. They are thoroughly discreditable, and in case new species are erected, are little less than criminal, considering the present overburdened and chaotic state of systematic bacteriology.

Thanks to the itch for species making, systematic mycology is generally cited as the most desperately confused and perplexing branch of natural science, but mycology is a highway turnpiked and provided with arc lights in comparison with the wilderness of systematic bacteriology. Of the thousand or

more forms which have been studied and named, or designated by letters or figures or vernacular names,[12] probably not one-tenth can be identified with any certainty owing to the meagerness of the descriptions. The older descriptions are particularly bad, and the effort to decide what was meant by these old names, for which somebody will by and by be strenuously claiming inalienable rights of priority, is usually time thrown away. There is quite enough to do in bacteriology, as every one knows who is dealing at first hand with its hard problems, without wasting precious energy in striving to guess what was meant by two and three line descriptions. All descriptions which do not *describe*, and there are many such, ought to be wholly ignored, and no species recognized as worthy of a place in literature unless so characterized as to be identifiable by others. A plea of this sort in the higher branches of botany or zoology would be a subject for laughter. Bad descriptions are however, so much the rule in bacteriology that it is no laughing matter but rather a great evil urgently demanding reform. It is a state of affairs which has come about naturally enough considering the way in which bacteriology has developed[13] but which would not now be tolerated for a moment in phanerogamic botany or in most branches of zoology and the continuance of which in bacteriology it is incumbent on every honest worker to limit and discourage in all possible ways. The best way in science, always, is to speak out plainly, and to join hands for the advancement of a good cause. Bad work should be ignored and "new species" relegated to limbo unless the descriptions conform to the requirements of modern bacteriological science, meaning by this expression the consensus of opinion among experienced and careful investigators everywhere. If there were some such agreement among the better class of workers, the improvement in systematic bacteriology would become very marked. The volume of publication would, indeed, decrease noticeably but this of itself

[12] About 650 species are mentioned in (***11***) Schizomycetaceæ, by de Toni and Trevisan in Saccardo's, *Sylloge Fungorum*, VIII, published in 1889, but this is not complete.

[13] All the early systematists built upon a foundation of sand, i. e. upon pure morphology.

would be a great advantage, and the quality of the work would more than correspondingly improve. Only in some such way can the strong tendency toward trashy publication be eliminated and light and order evolved from the present chaos.

With few exceptions, vegetable pathology seems to have been specially unfortunate in the class of persons who have devoted themselves to the study of bacterial diseases. While the bacterial side of animal pathology has had its Pasteur and Koch, its Esmarch, Hueppe, Flügge, Gaffky, Frænkel, Pfeiffer, Lœffler, Duclaux, Metchnikoff, Chamberland, Roux, Welch, Sternberg, Smith, Prudden, and a host of other skilled experimenters, scarcely less eminent, and has made correspondingly great progress, the study of the bacterial diseases of plants has been principally in the hands of botanists without special laboratory training in bacteriology and even destitute in some cases of an elementary knowledge of right methods of work. The great development of modern bacteriology is attributable largely to the discovery that human diseases are due to these organisms, and to its consequent alliance with medicine, but there is no reason why the same rigid scrutiny of methods and sharp calling in question of statements which have led to such brilliant results in animal pathology in recent years should not be applied in the same way to vegetable pathology. Accurate experimentation and trustworthy results are from a purely scientific standpoint quite as desirable in one field as in the other.

Two things are especially to be kept in mind in describing any bacterial disease of plants: (1) The pathogenesis must be worked out conclusively; (2) If the organism is named, it must be so described that it can be identified by any competent bacteriologist no matter where it is found.

The four requirements under the first head, i. e. *Pathogenesis,* are now generally recognized to be as follows:

A. Constant association of the germ with the disease.

B. Isolation of the germ from the diseased tissues and study of the same in pure cultures on various media.

C. Production of the characteristic symptoms of the disease by inoculations from pure cultures.

D. Discovery of germs in the inoculated, diseased tissues, re-isolation of the same, and growth on various media until it is determined beyond doubt that they are identical with the organism which was inoculated.[14]

Not one of these steps can be omitted. Possible sources of error beset the investigator at every step, and anything short of a rigid demonstration cannot be accepted as proof. A. is usually quite easy, involving only the careful microscopic examination of abundant material, stained and unstained. B. was made possible by the improvement of methods, *i. e.* by the use of solid media, and especially by the discovery of the method of isolation by means of plate cultures. C. is quite easy, provided the right organism has been obtained and this be inserted into the proper tissues under the right conditions to insure growth. The fulfillment, however, of these conditions often involves long and vexatious delays, and taxes the acumen of the investigator to the utmost. D. serves as a check on all the preceding work, showing that there has been no unintentional mixing of organisms, and that the results obtained were actually due to the supposed cause. For the sake of brevity these four rules of practice will be referred to in the following pages simply as A. B. C. and D. What weight shall be given any specific statement depends of course on the reputation of the individual. Some men are so careful of their reputation and so little given to making unwarranted statements that their simple word goes a long way even when the statements themselves seem improbable, whereas the elaborate explanations of other men, if the asserted facts are at all out of the ordinary, have to be taken with a grain of salt.

The requirements under the second head, i. e. *Description of the organism*, are more numerous, and are embraced under two general divisions of very unequal value, namely *Morphology* and *Biology*. In the classification of the higher plants and animals morphology has been accepted from time immemorial

[14] A series of successful reinoculations is always very desirable and becomes indispensable in case the infections are obtained on plants grown in a locality where the disease prevails naturally. Of course, numerous un-inoculated plants, known as "checks" or "controls," must always be reserved for comparison.

as answering all the requirements of systematists, but such is far from being the case when it comes to the description of bacteria. These minute organisms, which are among the lowest and simplest forms of living things yet discovered by man, are, within the commonly accepted generic limits, so morphologically similar as very often to be indistinguishable with any certainty even under the highest powers of the microscope. As supplemental, therefore, to morphology, and even in many cases as a complete substitute for it, we must have recourse to *Biology*, viz. to the behaviour of the living organism under a variety of known, artificially prepared conditions, such for example as the peculiarity of its growth on various culture media, its thermal death point, its ability to ferment various sugars, the chemical products of its growth, its pathogenic power, etc. Morphologically identical organisms often differ so widely and constantly in their biological peculiarities that there can be no question about their being distinct species, or as to the real value of this means of classification. Probably it also has value, hitherto overlooked, for the differentiation of higher plants and animals, especially for determining the limits of polymorphic or closely related species.

It is not my intention in this place to mention all the biological tests which should be applied to any species for its proper characterization. These are being added to constantly by an army of trained workers in all parts of the world, and my own views of what is at present necessary, or at least highly desirable, will be sufficiently evident in what is to follow. Very likely, also, as knowledge increases, some of the tests which are now generally held to be important will be shown to have little specific worth.

This, however, appears to be a good place to insist on accuracy in all the details of bacteriological work, especially in the preparation of culture media, and on explicitness of statement so that other investigators may know just what was done and how it was done, and thus be able to repeat the experiment. When all details of work are suppressed the inference, naturally enough, is that the writer was ignorant or else that he desired to conceal something not specially to his credit, and which if

plainly expressed might millitate against the value of his work. Either horn of the dilemma is equally bad. Some, however, who are desirous of doing good work in this field, or at least appear to be conscientious workers in other lines, do not seem to be aware of the necessity for extreme care in the preparation of culture media and the management of cultures. As a matter of fact, many bacteria are extremely sensitive to slight changes in the composition of the media in which they are grown or to other conditions within the control of the experimenter, and this appears to be true especially of the pathogenic species. Hence the many conflicting statements about the same organism. A few examples will render my meaning plainer. The careless exposure of cultures to bright sunshine may destroy the organism. An organism supposed to come from diseased tissues or from a culture, and which is being examined in a cover glass preparation, may have been derived actually from a contaminated staining fluid. The apparently simple matter of slightly unclean test tubes or flasks may lead to error, e. g. antiseptic influences may be at work, or preciptates may be thrown down and subsequently mistaken for bacterial growth. Some kinds of glass are unsuited to delicate bacteriological work, the culture fluids being contaminated by substances dissolved out of the walls of the beakers, tubes, and flasks. Tyros, of course, are liable to mistake almost anything for bacteria or to find them anywhere (See a long paper by Bernheim on (***12***) Die parasitären Bacterien der Cerealen, in *Münch. med. Wochenschrift*, 1888, pp. 743–745 and 767–770, and comments on the same by Buchner and Lehmann, *Ibid.*, 1888, p. 906, and 1889, p. 110). Boiling culture media, after it has been compounded, in open beakers or in loosely plugged test tubes or flasks may unwittingly lead to its concentration. The use at different times of different peptones, or grades of gelatine, of unlike per cents of gelatine or agar, of varying grades of acidity or alkalinity, of impure chemicals, of different concentrations of the nutrient media, and of different methods in its preparation all tend to render comparative studies impossible. A large source of error in the differentiation of species by means of sugar fermentation experiments has been the employment of bouillon

containing undetected muscle sugar. Even when preliminary tests are made with some gas-producing bacillus there is still an opportunity for error, provided the tests are carried on only for a day or two. No bouillon should be judged free from sugar and safe for use until in fermentation tubes it has been subjected for at least a week to the influence of *Bacillus cloacæ* or some other organism producing an abundance of gas from grape sugar. If at the end of this period no gas has developed, and the transfer of a loop of fluid from such a tube into another fermentation tube containing a dextrose-bouillon sets up an evolution of gas, then the first bouillon may be used with confidence. Again, if cane sugar is sterilized in an acid bouillon at least a part of it is *inverted*, i. e. changed into dextrose and fructose, and fermentation results obtained therefrom may be due to the presence of any one of three sugars. Bouillon should always be made distinctly alkaline before cane sugar is added. Many of the older fermentation experiments are worthless on account of neglect of such precautions, to say nothing of some recent ones. Again *Bacillus tracheiphilus* grows not at all or feebly on nutrient gelatine as ordinarily made, or in media which is acid beyond a determinable slight degree, and if only such media were used the erroneous conclusion might be reached that it could not be grown outside of the host plant, whereas it grows freely in artificial media, even on gelatine, when the right conditions are established. *Bacillus amylovorus* grows well in some gelatines and refuses to grow in others. Even comparatively slight changes in the acidity or alkalinity of the culture media often have a marked effect on the growth of certain organisms, while others, *e. g.*, *Bacillus cloacae*, are able to grow in almost any medium. Many bacteria prefer alkaline media, and some are very sensitive to the presence of acids, while a variety of bacteria commonly met with in water will not develop at all if the medium is rendered strongly alkaline. Other organisms grow well in acid media.[14a]

[14a] For a striking illustration of the effect on the growth of water bacteria of comparatively slight charges in the reaction of gelatine, see a recent table by George W. Fuller, in a paper entitled: (***13***) On the proper reaction of nutrient media for bacterial cultivation.—*Journal of the American Public Health Association*, Concord, N. H., Oct., 1895, p. 393.

Even the slightly varying acidity of steamed slices from different potato tubers may exert a marked effect on the growth of certain sensitive organisms. On this account some bacteriologists have advised discarding the potato altogether. I have myself found the potato a very useful substratum for the growth of both fungi and bacteria. All comparative tests on potato ought, however, to be made on cylinders or slices cut from the same tuber, and in every case the reaction, acid, neutral, or alkaline, should be carefully recorded. The behavior of the organism on a variety of tubers should also be determined, before deciding that it is something new. It has been thought by some that the best nutrient substance for a parasite must be, unquestionably, the juices of the host plant but this does not follow since there are all grades of parasitism, and even if it did, there are several chances for error in its employment, e. g. the nutrient juices are usually sterilized by steam heat and this may cause a number of chemical changes resulting in a compound very different from the living plant and entirely unsatisfactory as a culture medium, as many have learned by experience. Again, for some particular reason, even the juices of the plant when sterilized at ordinary temperatures by filtration, may be less well adapted to the needs of the parasite than well made beef bouillon or ordinary nutrient agar. In general, the standard culture media of bacteriology should be tried first. Some bacteria can be cultivated only on special media or at special temperatures, or under unusual conditions. *Bacillus subtilis* will only grow in the presence of free oxygen; *Bacillus amylobacter*, *B. tetani*, and *B. carbonis* will only grow in the absence of oxygen. Winogradsky states that his nitrifying organism obtained from European soils will not grow in the ordinary culture media and thrives best in solutions of inorganic substances, and on silicate jelly. *Bacterium tuberculosis* can be cultivated only in bouillon and on blood serum and nutrient glycerine agar, and at temperatures above 30°C. *Bacterium influenzæ* also flourishes at blood heat and can only be grown, it is said, in the presence of red blood corpuscles or in media containing yolk of eggs; other organisms have thus far refused to be cultivated at any temperature or on any artificial medium, e. g. *Bacterium lepræ* and *B. syphilitis.* Some bacteria

are destroyed at temperatures at which careless workers frequently pour their agar plates, while others refuse to grow at ordinary temperatures or even at blood heat, grow best at 50°–60°C., and are not killed until the temperature exceeds 70° or even 75°C. Finally, a race of *Bacterium anthracis* incapable of producing spores has been developed by growing the organism in media containing phenol; another non-virulent race bearing swollen, terminal spores, "drumsticks," by growing the organism in compressed air; and still another race destitute of virulence by cultivating it at temperatures above 40°C. These are not exceptional cases, similar care being necessary in all directions if one would avoid erroneous conclusions.

Naturally, every successful experimenter will vary his culture media in all sorts of ways in order to learn as much as possible of the organism under consideration, but at the same time he will determine its behaviour on the standard media, and will keep a very careful record of all that he does. The bacteriologist should make it an invariable rule to repeat every experiment two or three times, at the very least, and generally after an interval of some months or years he should repeat all his experiments. Even then he will fall into errors enough. He certainly should proceed with as much care as the chemist, and in many directions the work passes naturally over into chemistry. If quantitative or volumetric analysis requires all sorts of precautions and excess of care to avoid errors, no less does this youngest of all the sciences.

A few words respecting the occurrence of bacteria in normal plant tissues will be in place before concluding these general remarks. It goes without saying that such minute and universally distributed bodies as bacteria are likely to be found at times almost anywhere, even in plant tissues which seem to be healthy, just as they may sometimes occur in the blood stream of healthy animals, but they are not normally present in the tissues of plants. All carefully conducted experiments have led to this conclusion. The reader who wishes fuller information may consult papers by Laurent,[14b] Buchner,[15] Lehmann,[16]

[14b] (*14*) Sur la pretendue origine bacterienne de la diastase. *Bull. de l'Acad. roy. de Belgique*, T. X., pp. 38–57.

[15] (*15*) Notiz betreffend die Frage des Vorkommens von Bacterien in normalen Pflanzengewebe. *Muench med. Wochenschrift.*, 1888, pp. 906–907.

[16] (*16*) Erklarung in Betreff der Arbeit von Herrn Dr. Hugo Bernheim, etc. *Ibid*, 1889, p. 110.

Fernbach[17] Vestea,[18] Kramer,[19] and Russell.[20] Even when purposely introduced into living tissues they refuse to grow or spread but little and finally die out,[21] unless they possess specific pathogenic power in which case the result is quite different.

The diseases which will be discussed in the following pages may be divided into four classes:

(1). Diseases of clearly established bacterial origin.

(2). Diseases which appear to be constantly associated with bacteria and which are probably due to some specific organism, but full proof of which has not been furnished.

(3). Diseases said to be more or less closely associated with the presence of bacteria and ascribed thereto, but in which little or no proof has been brought forward to establish the causal relation.

(4). Communicable diseases which have been ascribed to bacteria but associated with which no organism has been found and which are probably of non-bacterial nature.

On the whole it would perhaps be more logical to divide the following pages into four chapters in the way I have specified, but for practical reasons it has seemed better to discuss all of the diseases of a given plant in one place. I have, therefore, arranged the material by hosts, but will at the close try to summarize the whole subject in the manner above indicated.

It will certainly be some time, probably many years, before we have anything like a permanent scheme of classification for the bacteria. Our knowledge is still too incomplete. Meanwhile, we have to do the best we can with the present systems, all of

[17] (***17***) De l'absence des microbes dans les tissus vegetaux. *Annales de l'Inst. Pasteur*, 1888, pp. 567–570.

[18] (***18***) De l'absence des microbes dans les tissus. *Ibid.*, 1888, p. 670–671.

[19] (***19***) Bakteriologische Untersuchungen ueber die Nassfäule der Kartoffelknollen. *Osterreichisches landw. Centralb.* I, Heft 1, 1891.

[20] l. c.

[21] Lominsky: (***20***) On the parasitism when introduced into plants of some disease-causing microbes (Russian). Wratch., 1890. No. 6, pp. 133–135.

Russell: l. c.

Kornauth: (***21***) Ueber das Verhalten pathogener Bakterien in lebenden Pflanzengeweben. *Centrb. f. Bakt., Parasiten-Kunde, u. Infectionsk.* I Abt., Bd. XIX, No. 21, 8 Juni, 1896, pp. 801–805.

which are more or less arbitrary and unsatisfactory, and all of which are liable to be set aside at any time. I have here adopted Migula's system[22] which seems to me very convenient, and on the whole the most satisfactory of any that has yet appeared.

Before proceeding to the body of this review it only remains to say that every effort has been made to deal impartially with the material in hand, and to present the essential ideas of the writers as concisely and accurately as possible. To this end the original papers have been consulted in every instance, unless otherwise stated in the text. So much vexation over wrong references has been experienced in time past by the writer that he has himself been at special pains to give full and accurate citations. It is to be hoped, therefore, that the reader will have no difficulty in finding the original papers. An endeavor has also been made to bring the subject fully up to date but it is quite likely that some worthy papers may have been overlooked, owing to the many languages and the ever increasing number of places of publication.

THE MEANING AND STRUCTURE OF THE SO-CALLED "MUSHROOM BODIES" OF THE HEXAPOD BRAIN.

By F. C. Kenyon, Ph. D.[1]

In looking at a series of sections of the brain of a hexapod, especially of a hymenopteron, the most notable structures are two pairs, one to each side, of large cup-shaped bodies of "Punkt substanz," or, what in the light of our present knowledge of nerve structure is better denominated fibrillar substance. Each of these cups is filled to overflowing with cells having large nuclei and very little cytoplasm. From the under surface

[22] Migula: Schizophyta: (22) Schizomycetes. *Die Natuerlichen Pflanzenfamilien* (Engler u. Prantl). I Teil. 1 Abt. a, Lief. 129. 8vo. p. 44, Leipzig, 1896. This is the forerunner of a larger work soon to be published by Gustav Fischer, Jena.

[1] Clark University, Mass.